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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

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0007637.2

29 MAR 2000

1. Your reference GBP82081

2. Patent application number
(The Patent Office will fill in this part)

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Pharma Mar, S.A.
Calle de la Calera 3
Poligono Industrial de Tres Cantos
Tres Cantos
28760 Madrid
Spain

4361141602

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

Spain

4. Title of the invention Antitumour compound

5. Name of your agent (if you have one)
"Address for service" in the United Kingdom to which all correspondence should be sent
(including the postcode)

Marks & Clerk
57 - 60 Lincolns Inn Fields
London WC2A 3LS

Patents ADP number (if you know it)

18001

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Country	Priority application No (if you know it)	Date of filing (day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support ofthis request? (Answer 'Yes' if:
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Continuation sheets of this form	0
Description	10
Claim(s)	
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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
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Request for substantive examination (Patents Form 10/77)	
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11. I/We request the grant of a patent on the basis of this application.

Signature Mark D Clark Date: 29 March 2000

12. Name and daytime telephone number of person to contact in the United Kingdom

GB Patent Filings
0207 400 3000

Document #: 504548

Antitumour compound

The present invention relates to antitumour compounds.

In particular, the invention relates to the anti-tumour compound ET-743, ET-743 delivery regimes, biochemical parameters that correlate with ET 743 treatment, treatment of soft tissue sarcoma and breast cancer with ET-743 and cells resistant to ET-743.

The invention includes a method of biochemical monitoring of a patient to identify a risk of multi-organ toxicity, MOT, when treated with Et-743. In one aspect, biliary biochemistry, bib, is monitored at entry to establish suitability for a proposed dosing regime. bib can be monitored during intercycles, with a view to dose reduction in the case of intercycle peaks of alkaline phosphatase above normal values.

The invention also includes a method of treatment of advanced soft tissue sarcoma, ASTS using Et-743. Again, bib is monitored.

Et-743 shows promise for patients with gastrointestinal stromal tumors, GIST; in non-GIST soft-tissue sarcomas, STS; and in non-GIST STS with no prior chemotherapy, that is, front line patients.

Furthermore, Et-743 is useful for treating patients advanced/metastatic breast cancer, A/MBC, who have been pre-treated, for example with anthracycline, A or taxane, T.

Other pretreated patients have benefited from Et-743, notably with sarcomas of bone and soft tissue.

We have additionally found that ET-743 has exceptional activity in the treatment of cancers, such as leukaemias, lung cancer, colon cancer, kidney cancer and melanoma.

Thus, the present invention provides a method of treating any mammal, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of a compound of the invention, or a pharmaceutical composition thereof. The patient may be one who has already received chemotherapy.

The present invention also relates to pharmaceutical preparations, which contain as active ingredient a compound or compounds of the invention, as well as the processes for their preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable composition or oral, topical or parenteral administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

Administration of the compound or compositions of the present invention may be by any suitable method, such as intravenous infusion, oral preparations, intraperitoneal and intravenous administration. We prefer that infusion times of up to 24 hours are used, more preferably 2-12 hours, with 2-6 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of say 2 to 4 weeks. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

The correct dosage of the compound will vary according to the particular formulation, the mode of application, and the particular *situs*, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The compound and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);

b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);

c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosphamide);

d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;

e) drugs which target topoisomerases such as etoposide;

f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;

g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;

h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplatin) or nitrosoureas;

i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;

j) gene therapy and antisense agents;

k) antibody therapeutics; and

l) other bioactive compounds of marine origin, notably the didemnins such as aplidine.

The present invention also extends to ET-743 for use in a method of treatment, and to the use of the compound in the preparation of a composition for treatment of cancer.

The invention is illustrated by the attached data.

CHARACTERIZATION OF IGROV-1 HUMAN OVARIAN CANCER CELL LINES MADE RESISTANT TO ECTEINASCIDIN-743 (ET-743). E. Erba, G. Di Liberti, I. Muradore, M. D'Incalci, G.T. Faircloth, and J. Jimeno. *Department of Oncology, Mario Negri Institute, Milan, Italy; PharmaMar USA, Inc., Cambridge MA, USA; and PharmaMar, S.A., Tres Cantos, Madrid, Spain.*

By exposing Igrov-1 human ovarian cancer cells to increasing concentrations of Ecteinascidin-743 (ET-743), for a short exposure time, we obtained sublines resistant to ET-743 which over-express Pgp. In the most resistant clone, Igrov-1/25 ET, the increased Pgp was not due to amplification of the mdr-1 gene but to increased mRNA levels. The IC_{50} values of ET-743 against Igrov-1/25 ET was approximately 50 times higher than the Igrov-1. Resistance was not reversed while maintaining Igrov-1/25 ET in drug free medium for at least 18 months. The cyclosporine analogue SDZ PSC-833 reversed the resistance of Igrov-1/25 ET to ET-743. Compared to Igrov-1 cells the Igrov-1/25 ET was cross-resistant to Doxo and VP16, equally sensitive to L-PAM, MNNG, CPT and only marginally less sensitive to Cis-DDP and Oxaliplatin. Igrov-1/25 ET exposed to Doxo retained this drug much less compared to Igrov-1. By exposing Igrov-1 cells to increasing concentrations ET-743 in combination with 1μM SDZ PSC-833 we have recently isolated a cell line resistant to ET-743. The IC_{50} values of ET-743 in the resistant line is approximately 5 times higher than in the parental cell line and the resistance is not reversed while maintaining the cell line in drug free medium for at least 3 months. The resistance is not related to overexpression of MDR-1 gene, nor to overexpression of MRP or LRP genes. The characterization of this differently resistant cell line is underway.

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IDENTIFICATION OF BIOCHEMICAL PARAMETERS THAT PREDICT THE ONSET OF SEVERE TOXICITIES IN PATIENTS TREATED WITH ET-743

Javier Gómez, Luis López Lázaro, Cecilia Guzmán, Ana González, Pharma Mar S.A., Madrid, Spain, Jean L. Misset, Chris Twelves, Angela Bowman, Klaas Hoekman, Michael Villalona, David Ryan, Luis Paz-Ares, ET 743 Phase I group, José Jimeno, Pharma Mar S.A., Madrid, Spain

ET-743 is a novel marine compound that is under active phase II development.The potential limiting toxicities, pancytopenia-fatigue, at the proposed recommended dose (RD) have been consistently characterized. However 6/331 patients (1.81%; 95%CI: 0.67%-3.90%) treated with ET-743 have developed severe or multiorgan toxicities (MOT) including long lasting pancytopenia, renal and hepatic failure and rhabdomyolysis; three out of those six cases died. Therefore full clinical and pharmacokinetic (PK) data from 93 phase I patients treated at the RD or at the maximal tolerable dose among five phase I trials have been included in a multivariate analysis to identify factors that can anticipate the onset of MOT in order to improve patients safety. Results: Patients with intercycle peaks (IPK) of alkaline phosphatase (ALP) above normal values (NV), median apex intercycle day=15, in a given cycle had a high risk of severe or MOT in the following cycle than those without ALP-IPK: 24% vs. 4.7% ($p<0.001$). In addition data from 108 cycles were included in a stepwise logistic regression model. The following variables remained as the main predictors of severe or MOT: ALP-IPK>1.1 NV p=0.0134, cycle baseline bilirubin >0.6 NV p=0.0042 and AST-IPK>5 NV p=0.0193.Other clinical, demographic and laboratory variables were not statistically significant. Moreover, an AUC>70 h.mcg/l correlates to both severe or MOT and ALP-IPK, raising the possibility that intercycle peaks of ALP are a marker of subclinical cholangitis yielding to decreased elimination of ET-743. Conclusions: these findings indicate the need to monitor the biliary biochemistry (bib) at entry and during the intercycle period, performing one bib test within intercycle days 14-17. Patients with normal bib at baseline can be safely treated with the proposed RD of 1500 mcg/m2. A dose reduction to 1200 mcg/m2 is mandatory in case of ALP-IPK. A prospective identification of the RD in patients with impaired bib is warranted.

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Abstract # 101414 has been Submitted

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PHASE II STUDY OF ET-743 IN ADVANCED SOFT TISSUE SARCOMA (ASTS) IN ADULT : A STBSG-EORTC TRIAL. LE CESNE A, JUDSON I, BLAY JY, RADFORD J, VAN OOSTEROM A, LORIGAN P, RODENHUIS S, DONATO DI PAOLA E, VAN GLABBEKE M, JIMENO J AND VERWEIJ J. STBSG AND EORTC DATA CENTER, BRUSSELS, BELGIUM

Axel Le Cesne, Institut Gustave Roussy, Villejuif, France

Antitumor activity of Ecteinascidin (ET-743) (Pharma Mar / Spain) in ASTS has been reported in previous phase I studies. The main objectives of this phase II study were to assess activity and toxicity of ET-743 administered at a dose of 1500 [illegible]g/m2 as 24-hour continuous infusion every 3 weeks in patients (pts) with ASTS. Pts with non-gastrointestinal stromal tumors (GIST) ASTS (group A) received ET-743 as 2nd line chemotherapy (CT) while pts with GIST (group B) received it as front-line therapy. Pharmacokinetic analyses were planned during the 1st and late cycle (cy). Results : to date, 54 pts have been included (39 in gp A and 15 in gp B) with a median age of 57 yrs (18 to 76 yrs) and a median PS of 1 (0 to 1). One third of gp A pts had leiomyosarcoma. Toxicity (T) : febrile neutropenia, NCI-CTC gde 3-4 neutropenia (ANC) and thrombocytopenia were observed in 10, 48 and 28% of pts respectively. Haematological T was mainly observed after the 2nd cy (21% of gde 4 ANC vs 3% after the 1st cy). A reversible transient elevation of transaminases was seen in the majority of cy. There were 3 toxicity-related deaths, 2 due to neutropenic fever, renal insufficiency and liver failure, and 1 due to neutropenic sepsis with an unusual elevation of creatine kinase. A creatinine elevation occurred in 2 other pts in the first 2 cy, 1 of whom with previous nephrectomy required transient haemodyalisis. There was no alopecia and no severe GI T. The severe T were highly correlated with 1) an abnormal alkaline phosphatase (ALP) baseline value of and 2) a rise of ALP and/or bilirubin (bil) between cy. A protocol amendment now requires normal ALP at inclusion and ET-743 dose reductions (1200 [illegible]g/m2) in case of an intercycle-rise in bil/ALP > grade 1. Early hints of activity have been observed and final results will be presented.

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ECTEINASCIDIN (ET-743) SHOWS PROMISING ACTIVITY IN DISTINCT POPULATIONS OF SARCOMA PATIENTS: SUMMARY OF 3 U.S.-BASED PHASE II TRIALS.

Demetri GD, Seiden M, Garcia-Carbonero R, Supko J, Harmon D, Goss G, Merriam P, Waxman A, Quigley MT, Jimeno J, Ryan D. Dana-Farber Cancer Institute and Massachusetts General Hospital (Dana-Farber/Partners CancerCare), Boston MA and PharmaMar, Inc, Cambridge MA

ET-743, a cytotoxic alkaloid derived from a marine organism, covalently binds to the minor groove of DNA and may inhibit DNA replication and transcription through other mechanisms. Prior *in vitro* and phase I studies have documented potent cytotoxic activity of ET-743 in various tumors of mesenchymal origin. Based on these data, coordinated phase II clinical trials have been designed to assess the activity of ET-743 in distinct groups of patients (pts) with sarcomas. Gastrointestinal stromal tumors (GIST), rigorously defined as GI spindle cell tumors overexpressing c-*kit*, were entered into one trial. A second study evaluated pts with non-GIST soft-tissue sarcomas (STS) who had received 1 or 2 prior CT regimens for metastatic disease. Another study is the first ever to use ET-743 in non-GIST STS pts with no prior CT (front-line study). The dose and schedule of ET-743 administration (1500 $\mu g/m^2$ by 24-hr continuous IV infusion on an outpatient basis, repeated every 3-4 weeks) were identical across all studies. Pts were restaged after every 2 cycles. Accrual to all studies from 8/99 to 11/99: GIST = 8 pts; Prior CT = 16 pts; Front line = 12 pts. Tolerability of treatment has been very good. Nausea has been essentially eliminated by incorporating dexamethasone into a prophylactic antiemetic regimen. Other toxicities were similar to those observed during phase I studies, such as myelosuppression, temporary and asymptomatic transaminitis, and fatigue. 6 of 10 evaluable pts (60%) in the upfront study have exhibited stable disease or minor response after 2 cycles of therapy, as have 4 of 12 evaluable pts (33%) with prior CT. Preliminary evidence of clinical activity has been observed in liposarcoma, leiomyosarcoma, and synovial sarcoma. No responses have yet been observed in 5 evaluable pts with GIST. Pharmacokinetics (PK) of ET-743 are monitored in all pts during the first cycle of therapy to assess interpatient variability and possible correlations between PK and clinical activity or toxicity. We conclude from these ongoing trials that ET-743 represents an extremely promising agent for the management of several histologic subtypes of STS.

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ECTEINASCIDIN-743 (ET-743) IN TAXANE (T)/ANTHRACYCLINE (A) PRETREATED ADVANCED/METASTATIC BREAST CANCER (A/MBC) PATIENTS (pts): PRELIMINARY RESULTS WITH THE 24 HOUR (h) CONTINUOUS INFUSION (CI) Q3WEEK SCHEDULE. L. Zelek; A. Yovine, E. Brain, J. Jimeno, A. Teamma, C. Martin, M. Spielmann, E. Cvitkovic, JL. Misset. Inst. Gustave Roussy, Villejuif; Centre René Huguenin, Saint Cloud; Hôp. P. Brousse, Villejuif; C&AC, Kremlin-Bicêtre, France. PharmaMar, Madrid, Spain.

ET-743 is a new minor groove DNA-binding agent of marine origin, specific to guanine-cytosine regions, active in a wide variety of murine tumors and human xenografts, including breast cancer. It is in late phase I and early phase II development. We report our clinical experience with ET-743 given q3weeks as 24h CI in pretreated A/MBC pts. We analyze 14 pts treated in an ongoing phase II study (1500µg/m²), 4 pts treated at the same dose and 2 at 1800µg/m² (MTD) treated in the phase I study (E. Cvitkovic, et al. ASCO 99, Abst.690). Patients: 20 women, all with measurable disease and progressing at study entry, median (m) age 50 years (33-64), m PS (ECOG) 0 (0-1), m nb of involved organs: 2 (1-4). Disease sites: cutaneous 12 (60%), liver 10 (50%), bone 9 (45%), lymph nodes 6 (30%), pleuropulmonary 6 (30%); m number prior regimens: 2 (1-6). All pts and 16 were A and T pretreated, respectively. Five pts were resistant to both A and T, 3 to A only and 2 to T only. Safety: 56 cycles (cy) were administered, m 2 cy/pt (1-8). Grade 3/4 (NCI) toxicities per cy: neutropenia 25/50%, thrombocytopenia 4/2%, reversible transaminitis 34/0%, asthenia gr 2/3 in 13/2%. There were no differences with the safety profile of the phase I experience. Efficacy: As of 11/99, 16 pts are evaluable (4 pts too early). Two partial responses (13%, 95% CI:2-38%) were observed (pleuropulmonary, thoracic skin involvement) lasting 3.5 and 2+ months, both without primary resistance to A or T; 6 pts achieved disease stabilization (2+, 3, 3, 3+, 4.5, 6+), including 2 with sustained CA 15-3 decrease (40% decrease 1pt; normalization 1pt). ET-743 shows early evidence of clinical activity in heavily pretreated A/MBC. Accrual is ongoing and further phase II trials are warranted.

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PRELIMINARY EVIDENCE OF ACTIVITY OF ECTEINASCIDIN (ET-743) IN HEAVILY PRETREATED SARCOMAS OF BONE AND SOFT TISSUE (STS) PATIENTS (pts).

S. Delaloge, A. Yovine, A. Taamma, P. Cottu, M. Riofrio, E. Raymond, E. Brain, M. Marty, J. Jimeno, E. Cvitkovic, J.L. Misset. Hop. P. Brousse, CAC, Hop. Saint Louis. Inst. Gustave Roussy, C. René Huguenin, France ; Phar Mamar. Spain.

ET-743, a tetrahydro-isoquinolone of marine origin, has demonstrated high preclinical activity against various solid tumor cell lines and xenografts. Neutropenia (AGC) and thrombocytopenia (T) were the limiting toxicities in our 24h CI-schedule phase I trial (E. Cvitkovic et al. ASCO 99, Abst.690). Activity against sarcomas was observed, and prompted the initiation of phase II trials. Pts not eligible for phase II were treated on a compassionate use basis. We report here our overall current experience in refractory, advanced sarcoma pts. **Patients:** Since 2/98, 39 pts (35 STS, 3 osteosarcoma, 1 Ewing) have received ET-743 as 24h-CI q3weeks, either at the recommended dose of 1500 mcg/m² (37 pts), at MTD (1800µg/m², 1 pt), or at 1200µg/m² (1 pt). 9 pts were treated in the phase I trial, 15 in the ongoing phase II trial, 15 on a compassionate basis. 22 female, median (m) age 45 (16-71), m PS 0 (0-2). 22 pts had bulky disease at initiation. All pts had previously received anthracyclins (A) and alkylators. M number of prior chemotherapy lines 2 (1-7). 56% disease progression under the prior chemotherapy regimen. **Results:** Toxicity is evaluable for the 137 given cycles (median 2 cy/pt, 1-12+). NCI grade 3-4 toxicities are AGC (34% ; with 6.5% febrile), T (5%), and acute reversible transaminitis, as previously described (44%). Efficacy: 34 pts are evaluable (5 too early). There were 4 PR (11.7%), 2 being converted to post-surgical CR, 3 MR (1 ongoing, 1 post-surgical CR), 11 SD, most lasting ≥3 mo. Responses were seen in various histologic types (including 2/3 osteosarc.), in all disease sites (including visceral metastases), in bulky and non-bulky disease, and A-refractory or not refractory tumors. **Conclusion:** ET-743 appears as a promising new agent in heavily pretreated bone and STS pts.